

Improvements in the Rearing of the Tachinid Parasitoid *Exorista larvarum* (Diptera: Tachinidae): Influence of Adult Food on Female Longevity and Reproduction Capacity

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Subject Editor: Muhammad Chaudhury

Received 23 August 2018; Editorial decision 2 November 2018

Abstract

Exorista larvarum (L.), a polyphagous gregarious larval parasitoid of lepidopterans, can be mass produced both in vivo, using the greater wax moth *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) as a factitious host, and in vitro, on artificial media composed of crude components. The present study was focused on another aspect of *E. larvarum* rearing, namely the influence of adult food on parasitoid performance. The standard food, consisting of lump sucrose and cotton balls soaked in a honey and water solution (1), was compared with other foods or food combinations, namely lump sucrose alone (2), honey and water solution (3), sucrose and water solution either alone (4) or combined with bee-collected pollen (5), and, finally, pollen alone (6). All foods were provided together with distilled water supplied in drinking troughs. Based on the parameters considered (i.e., female longevity, number of eggs laid on host larvae, puparia obtained from eggs, and adults emerged from puparia), pollen alone was deemed to be the most suitable food for adult females of *E. larvarum*. In particular, the pollen showed a longevity-promoting effect, increasing the number of eggs laid on host larvae throughout the female lifespan. The use of this adult food may also result in a higher flexibility of the management of *E. larvarum* colonies because it can be replaced weekly, as no desiccation or mold infections were ever found to occur.

Key words: Tachinidae, parasitoid, insect rearing

Tachinid parasitoids, which include about 8,500 species (O'Hara 2013), are far less studied than parasitic hymenopterans. Yet, they play a major role in regulating the populations of herbivore insects, primarily larval lepidopterans, which represent 70% of their known host species. Tachinidae represent the largest and most important group of nonhymenopteran parasitoids and are notable because of their diverse oviposition strategies that allow them to parasitize hosts in varying environments. For example, many tachinids are unique because of their capacity to attack hosts hidden in the soil or in plants (Dindo and Grenier 2014).

The subject of the present study, *Exorista larvarum* (L.), is a polyphagous, gregarious larval parasitoid of lepidopterans. This tachinid is a Palearctic species, but throughout the 20th century, it has been repeatedly introduced in North America in classical biological control programs against the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Erebididae) and is now established in the areas of introduction (Sabrosky and Reardon 1976, Kenis and Lopez Vaamonde 1998). Previous studies reported on a variety of aspects of *E. larvarum*, including the biology, morphology, and anatomy of its larval stages (Michalková et al. 2009, Valigurová et al. 2014), host

selection, and oviposition strategies (Dindo and Nakamura 2018). Briefly, the females lay macrotype eggs on the host body, and the newly hatched larvae penetrate the host integument, build integumental respiratory funnels, and continuously develop until pupation, which generally occurs outside the host remains.

Exorista larvarum can be mass produced relatively easily both in vivo, using larvae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), as a factitious host, and in vitro, on artificial media composed of crude components (Dindo and Grenier 2014). This parasitoid may thus be considered as a good candidate for augmentation, as the success of this type of biological control is largely based on the development of efficient techniques for culturing the entomophagous species involved (Greathead 1986). Moreover, *E. larvarum* production may also be useful on a small scale for laboratory studies evaluating, for instance, the impact of insecticides on this nontarget species (Marchetti et al. 2009, Francati and Gualandi 2017). Research aimed at optimizing the rearing techniques of this parasitoid is thus justified. The results of two studies on this topic were presented by M. L. Dindo and P. De Clercq at the 14th IOBC-MRQA Workshop held in Mérida (Mexico) from 14 to

17 November 2017 (Dindo and De Clercq 2017). Both studies dealt with the possibility to improve the in vitro rearing of *E. larvarum*, either by supplementing the medium with hemolymph of nonpermissive hosts (Dindo et al. 2016) or by storing in vitro-cultured eggs or larvae at suboptimal temperature to improve colony management and build up a stock of flies (Benelli et al. 2017b).

The present study focused on another aspect of *E. larvarum* rearing, namely the influence of adult food on the performance of the parasitoid. Although the nutritional requirements of adult parasitoids (whether hymenopterans or tachinids) are poorly understood, it is known that most of them feed on nectar or honeydew. These food sources, which are rich in carbohydrates, provide their energetic needs (Thompson and Hagen 1999), and according to Wäckers (2003), nectar might also contribute to egg maturation and other physiological processes. In nature, tachinids may play a role as pollinators (Griffin et al. 2009, Al-Dobai et al. 2012) and may also feed on pollen (Campadelli 1977). Many flowering plants are reportedly visited by European tachinids, mainly (but not exclusively) Apiaceae (Mellini 1991).

Like many other aspects of parasitoidism, adult nutrition has been investigated mostly for hymenopteran parasitoids. For example, honey bee products, especially royal jelly completed with honey or pollen grains, were reported to be a suitable food for the adults of the ectoparasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae), as they increased parasitoid fecundity, egg hatch rate, and longevity in comparison with a sugar solution (El-Wahab et al. 2016). In general, fecundity, egg hatch rate, and longevity, together with adult emergence, were considered an important tool to explore the effects of food, especially carbohydrate resources, on adult parasitoid wasps (Liu et al. 2015, Bari et al. 2016, Harvey et al. 2017).

A few studies were especially devoted to the influence of adult food on the performance of tachinid parasitoids. In captivity, adult tachinids are often provided with lump sucrose and/or honey solutions, which may be gelled or supplied through cotton balls (Mellini and Coulibaly 1991, Quednau 1993, Dindo and Grenier 2014). Also, in the stock colony of *E. larvarum* maintained at the Department of Agricultural and Food Science (DISTAL; University of Bologna, Italy), the captive flies were routinely fed on lump sucrose and cotton balls soaked in a honey and water solution, but the effects of adult food had not been studied in detail. Therefore, the present study was aimed at comparing the effect of different types of adult foods on the longevity and reproductive capacity of *E. larvarum*. The latter parameter was assessed by counting the numbers of eggs laid on host larvae, puparia obtained from those eggs (which reflect fertility, Dindo et al. 2006), and adults emerged from puparia. These parameters were selected among those suggested by Grenier and De Clercq (2003) to assess the quality of parasitoids produced under artificial conditions. A practical objective was to simplify the colony management by reducing labor, considering that the honey solution-soaked cotton balls need to be changed every 2–3 d, due to desiccation and contamination by mold.

Materials and Methods

Insects

A laboratory colony of *E. larvarum* was established in 2004 from adults, which had emerged from *Hyphantria cunea* (Drury) (Lepidoptera: Erebididae) larvae field collected in the province of Modena (44° 10' 49" N, 10° 38' 54" E; Emilia Romagna, northern Italy). The colony was maintained in laboratories of DISTAL using *G. mellonella* as a host. *Galleria mellonella* larvae were reared on an

artificial diet (Campadelli 1987) and were kept in complete darkness at $30 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH. Parasitization occurred by exposing last instar larvae (the most suitable stage according to Hafez 1953 and Dindo et al. 2003) to *E. larvarum* females for about 1 h.

The adult parasitoids were kept in Plexiglas cages ($40 \times 30 \times 30$ cm) at $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH, and a photoperiod of 16:8 (L:D) h (50–70 flies per cage). The adults were fed two to three pieces of lump sucrose per cage, each weighing 3 g on average, and two to three cotton balls soaked in a honey and water solution (20% honey w/w); the final weight of the soaked balls ranged between 20 and 25 g each. This has been used for a long time (i.e., about 30 yr) as a standard food in our laboratories to feed adults of *E. larvarum* and other tachinid species (Mellini and Coulibaly 1991, Dindo and Grenier 2014). The pieces of sucrose were changed every week, but soaked cotton balls had to be changed three times a week because they dried quickly and were easily infected by molds. Water (very important for tachinid flies) was provided via drinking troughs (capacity 150–200 ml) consisting of cotton plugs moistened with distilled water contained in a small plastic reservoir. Drinking troughs were changed weekly.

Comparison of Different Adult Foods

Six adult foods, each corresponding to a treatment, were compared. The foods were as follows: 1) the standard food, consisting of one 20% honey solution-soaked cotton ball and one piece of lump sucrose (control treatment); 2) lump sucrose alone (one piece), similar to previous studies with other tachinid adults, such as *Exorista mella* Walker (Diptera: Tachinidae) (Adam and Watson 1971) and *Zenillia dolosa* (Meigen) (Diptera: Tachinidae) (Ho et al. 2011); 3) one honey solution-soaked cotton ball (20% honey w/w), as reported by Sourakov and Mitchell (2002) for *Chetogena scutellaris* (Wulp) (Diptera: Tachinidae); 4) sucrose and water solution (33% sucrose w/w) administered via drinking troughs similar to those used to supply water; this solution was intended to resemble the nectar sugar concentration in nature, which is often sucrose dominated (Chalcoff et al. 2006, Wolff 2006, Somme et al. 2016), whereas the 33% concentration was selected because it is in between the concentrations found in nature, which are highly variable, ranging from about 5 to 75% (Abrol 2012, Knopper et al. 2016); 5) sucrose and water solution (33% sucrose w/w) as mentioned earlier, combined with pollen (3 g), which was added as a source of proteins and amino acids (Nicolson and Human 2013, El-Kazafy et al. 2017) and carbohydrates (Pacini et al. 2006); and 6) pollen alone, supplied as described earlier. In treatments (5) and (6), corbicular honeybee pollen was used. It was obtained from an apiary located in a natural area in the surroundings of Bologna. A commercially available pollen trap, placed in front of the hive, was used to dislodge the pollen pellets from the corbiculae of returning foraging honeybees. The fresh pollen pellets were collected and stored in a freezer at -18°C . The pollen pellets were homogenized and placed on the bottom of a 3-cm-diameter plastic Petri dish to be supplied to *E. larvarum* adults. Palynological analysis, based on the morphological assessment of the pollen grains (Persano Oddo and Ricciardelli D'Albore 1989), showed that the pollen was mainly composed of *Populus* sp.

For every adult food (= treatment), 10 replicates with a single female each were run. Each female was paired with a male. All adults were obtained from puparia of uniform weight (35–45 mg) and were newly emerged (<24 h). Each pair was placed inside a $20 \times 20 \times 20$ cm Plexiglas cage, with a drinking trough with distilled water like those utilized in the standard rearing. As in the standard rearing, the honey solutions were changed three times a week,

whereas the other food sources and distilled water in the drinking troughs were changed weekly.

As in *E. larvarum* mating occurs soon after emergence, and the preoviposition period lasts 2–3 d at 25–26°C (Dindo et al. 2007), in all treatments, the males were removed from the cages 3 d after pairing, and from the third day, the females were daily supplied with final instar *G. mellonella* larvae (three per female) until death. Larvae were exposed to parasitoid females for 1 h, and after counting the eggs on their body, they were placed in 6-cm-diameter plastic cups until puparium formation. The newly formed parasitoid puparia were counted and transferred singly into glass vials until adult emergence. The adults were also counted. The experiment was conducted at $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH, and a photoperiod of 16:8 (L:D) h.

Parameters Evaluated

Effects of adult foods on longevity and reproduction of *E. larvarum* were evaluated in terms of the following parameters: 1) female longevity from emergence (in days); 2) number of eggs per female laid on host larvae during the first 10 d from the onset of oviposition (when most eggs are laid according to Dindo et al. 1999) (E10) and throughout the female lifespan (E). These parameters were calculated by daily counting the number of eggs per female (e) laid on host larvae (Dindo et al. 1999); 3) eggs that produced puparia (%) calculated based on the number of eggs laid throughout female lifespan; and 4) adult emergence (%) calculated based on the number of puparia obtained.

Statistical Analysis

The data for female longevity, eggs laid in 10 d (E10) and throughout female lifespan (E), and adult emergence were analyzed by one-way ANOVA. In case of significant differences ($P < 0.05$), means were compared by Tukey's honestly significant difference (HSD) multiple-range test. The percentages of eggs that produced puparia were analyzed by a Kruskal–Wallis test because of heterogeneity of variances. An arcsine transformation (Mosteller and Youtz 1961) was used to transform percent values for analysis. Statistical tests were performed with STATISTICA 10.0 (Statsoft Inc. 2010).

Results

Female longevity was significantly influenced by the adult food source ($F = 4.04$; $df = 5, 54$; $P = 0.003$). The females fed on sucrose or on pollen lived the longest (means \pm SE: 25.9 ± 3.7 d and 29.2 ± 2.3 d, respectively), whereas those fed on 20% honey solution were the

most short lived (12.9 ± 1.9 d; Fig. 1). For the number of eggs per female laid on host larvae in 10 d (E10), no significant difference was found among the adult food sources ($F = 0.48$; $df = 5, 54$; $P = 0.78$). The differences in longevity, however, influenced the number of eggs laid per female throughout female lifespan (E), which was also significantly affected by adult food ($F = 4.58$; $df = 5, 54$; $P = 0.0014$). The longest longevity resulted in the highest E values ($= 207.2 \pm 18.9$ for the females fed on pollen alone and 144.2 ± 17.5 for the sucrose-fed females; Fig. 2). No significant differences were found among the different adult foods for the percentages of eggs that produced puparia and for adult emergence. It must be emphasized, however, that in all treatments except pollen no puparia were formed in some replicates (Table 1).

Discussion

In the rearing of insect parasitoids, little attention is usually given to the selection of the adult parasitoid food source in captivity, based on the assumption that any sugar-rich substrate may be suitable. More consideration should, however, be given to this topic because parasitoid life traits can be affected by different types and concentrations of nutritional resources, and adult nutrition may thus play a pivotal role in biological control programs (Jervis et al. 1992, Wäckers 2003, Benelli et al. 2017a).

Based on the parameters considered in our study, the standard food routinely supplied to *E. larvarum* adults since the establishment of our laboratory colony (20% honey solution and lump sucrose) was less efficient than some of the other foods tested, especially pollen alone. Pollen showed a longevity-promoting effect on *E. larvarum* females and had a positive influence on the number of eggs laid on host larvae throughout the female lifespan. As already stated, tachinids may feed on pollen in nature (Campadelli 1977), but no record has been found in the literature of pollen supplied as food for either *E. larvarum* adults or other tachinid flies in captivity. Pollen has, however, been used as a food source for captive hymenopteran parasitoids, either alone or in combination with other carbohydrate sources (Zhang et al. 2010).

A palynological assessment revealed that the main component of the corbicular pollen pellets from honeybees used here was *Populus* pollen, which was classified as “good” according to protein content (23.98%) and contribution to the nutrition of honeybees (Liolios et al. 2015). In general, pollen also contains carbohydrates (mainly glucose, fructose, and sucrose; Pacini et al. 2006). In corbicular pollen, which is moistened by foraging bees with nectar and

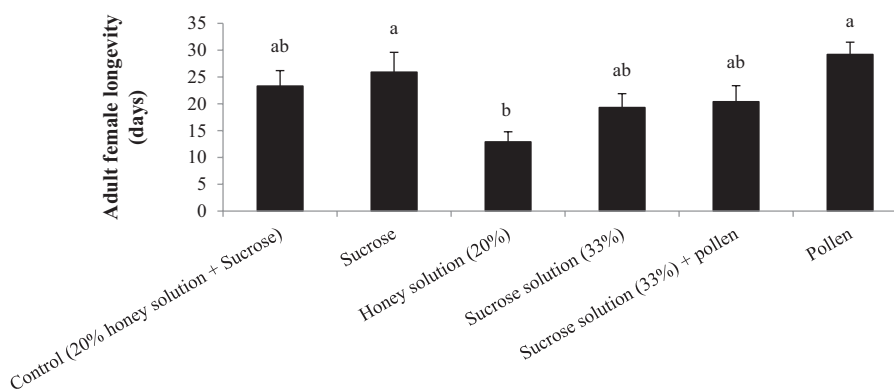


Fig. 1. Longevity from emergence (in days) of *Exorista larvarum* females fed as adults with different food sources. Different letters above the columns indicate differences in the mean longevity (as determined by one-way ANOVA followed by Tukey's multiple-range test). Error bars indicate SEM. Number of replicates per treatment = 10, each comprising one female. See text for statistical results.

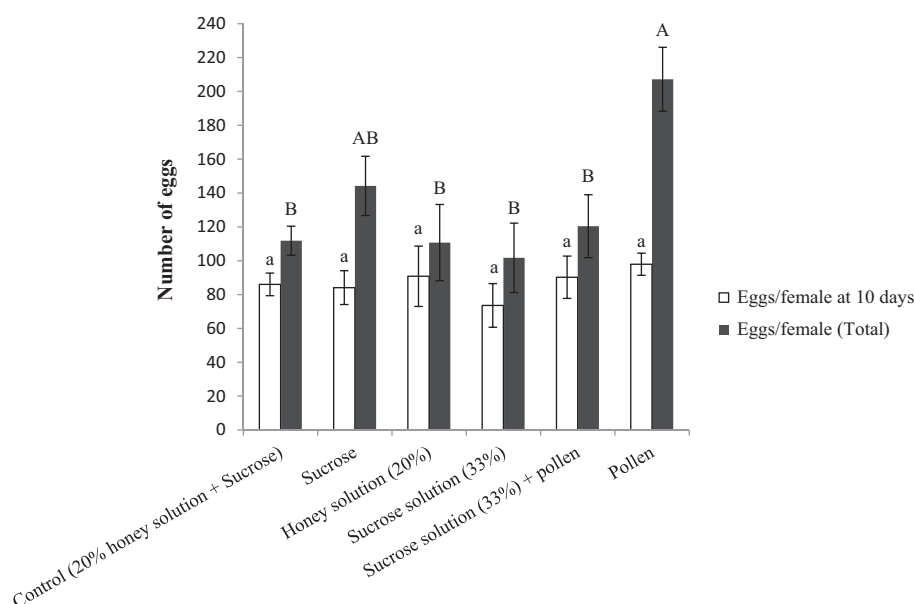


Fig. 2. Influence of adult food on the number of *Exorista larvarum* eggs per female laid on host larvae in 10 d following the beginning of oviposition (E10; white columns) and throughout female lifespan (E; black columns). Different letters above the columns indicate differences in the mean E10 values (lowercase) and E values (uppercase) as determined by one-way ANOVA followed, for E, by Tukey's multiple-range test. Error bars indicate SEM. Number of replicates per treatment = 10, each comprising one female. See text for statistical results.

Table 1. Eggs laid throughout *Exorista larvarum* female lifespan (E) that produced puparia (%) and adult emergence (%)

Adult food	Eggs that produced puparia (%) ¹	Adult emergence (%) ²
Control (20% honey solution + sucrose)	23.3 ± 5.6a (10)	93.3 ± 2.3a (7) ³
Sucrose	22.3 ± 3.1a (10)	90.2 ± 2.3a (9) ⁴
Honey solution (20%)	23.3 ± 5.6a (9) ⁵	91.5 ± 2.4a (9)
Sucrose solution (33%)	16.8 ± 6.1a (10)	94.9 ± 1.7a (7) ³
Sucrose solution (33%) + pollen	23.3 ± 5.6a (9) ⁵	92.0 ± 2.0a (7) ⁶
Pollen	25.6 ± 2.1a (10)	93.6 ± 1.8a (10)
F (df)		0.26 (5,43)
H (N)	4.4 (58)	
P	0.49	0.93

Means in a column followed by the same letter are not significantly different ($P > 0.05$) according to a Kruskal–Wallis test (eggs that produced puparia) or one-way ANOVA (adult emergence). Numbers of replicates are given in parentheses after the means (\pm SE).

¹Percentages calculated over the number of eggs laid throughout female lifespan.

²Percentages calculated over the number of puparia.

³Only seven replicates were considered because in three replicates, no puparia formed, and thus no adults emerged.

⁴Only nine replicates were considered because in one replicate, no puparia formed, and thus no adults emerged.

⁵Only nine replicates were considered because in one replicate, no eggs were laid, and thus no puparia were obtained.

⁶Only seven replicates were considered because in two replicates, no puparia formed, and thus no adults emerged.

glandular secretions, the amounts of sugars are even higher than in pollen collected directly from flowers (Conti et al. 2016). The carbohydrate content and composition of the pollen used in this study was not determined and should be the topic of further research. The results achieved, however, suggest that this pollen was better than the standard food for the nourishment of captive *E. larvarum* flies. Considering that protein and sugar content may vary noticeably among pollens, depending on different factors including plant species (Roulston and Cane 2000), future studies will evaluate the effects of diverse types of pollen on the longevity, reproductive capacity, and other quality traits of *E. larvarum* adults.

Moreover, unlike the honey solution-soaked cotton balls, which must be changed at 2- to 3-d intervals, pollen may be replaced weekly (or, if need be, even at longer intervals), as no desiccation or mold infections were found to occur during our experiments. Based

on the results achieved in this study, the standard food in the *E. larvarum* colony maintained at DISTAL, which included honey-soaked cotton balls, has now been replaced by honeybee pollen, in addition to the distilled water supplied in drinking troughs.

Among the other foods tested, it is noteworthy that the 33% sucrose solution (intended to resemble nectar) proved unsuitable either alone or even in combination with pollen. The sucrose solution may not have offered sufficient nutrients because a better adult performance was obtained with lump sucrose alone, in terms of longevity and eggs laid throughout female lifespan. We do not have a clear explanation for the lower longevity and number of eggs laid by the female flies fed on sucrose solution combined with pollen. A hypothesis to be verified could be an increase of humidity in the small experimental cage due to the presence of both the sucrose solution and the distilled water in drinking troughs. A negative physical

effect of the stickiness of the sucrose solution on the flies, resulting in more time and energy spent grooming, might also be responsible for the lower performance on this type of food.

Further research is necessary to address the different issues that arose during this study and to test the effects (also intergenerational) of these and other food combinations on longevity, reproduction capacity, and other quality parameters of *E. larvarum* adults. In particular, considering the great variability of the composition of pollen in nature, also in relation with its dispersion type (Dobson 1988), it is worth investigating the influence of different pollens on the performance of *E. larvarum* and other tachinid parasitoids. The results obtained so far have, however, already helped to improve the laboratory rearing of this tachinid species and, at the same time, may constitute a basis for carrying out similar studies with other parasitoid species.

Acknowledgments

We are grateful to Elisa Marchetti for her valuable contribution during the experiments and to Fabio Sgolastra for his useful suggestions and comments. This research was supported by the University of Bologna (RFO14DINDO). The Ministry of Science, Research and Technology of the Islamic Republic of Iran supported M.R.'s internship at the Department of Agricultural and Food Science (DISTAL) of the University of Bologna.

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